THE BEHAVIOR AND METAMORPHOSIS OF THE LARVA OF BUGULA NERITINA (LINNAEUS): EXPERIMENTAL MODIFICATION OF THE LENGTH OF THE FREE-SWIMMING PERIOD AND THE RESPONSES OF THE LARVAE TO LIGHT AND GRAVITY

WILLIAM F. LYNCH

University College, New York University

McDougall (1943) noted that the larvae of *Bugula neritina*, when liberated in finger-bowls containing sea water at room temperature (25–30° C.), swam without exception towards the source of illumination and within an hour the majority became attached to the surface film or to the sides of the container. The larvae remained positively phototropic throughout their pelagic stage. While experimenting with light boxes containing chambers arranged at the same horizontal level but receiving different amounts of illumination, however, he found 1,533 settings in the two chambers receiving the least amount of illumination, but only half as many in the two most brightly lighted ones. Furthermore, on tiles submerged in Beaufort Channel the most intense vertical distribution of attached larvae was very near the bottom at a depth of 79–104 inches. Their numbers gradually decreased up to a point 6 inches below mean low watermark; presumably the inability of *Bugula* colonies to tolerate exposure to air for more than a few minutes accounts for their absence above this level. These observations indicate that larvae in their natural environment become photonegative and geopositive some time before attachment. McDougall (1943) called attention to the marked contrast between the behavior of the larvae under laboratory conditions and their apparent behavior in nature.

The studies reported in this paper were undertaken to determine what environmental factors may be responsible for the observed distribution of adult colonies at Beaufort, North Carolina. Incidentally they led to an investigation of the mechanisms involved in causing metamorphosis.

The general literature on the development and metamorphosis of the Bryozoa is fairly extensive. A monograph by Barrois (1877), reviewing the behavior, development and metamorphosis of representative types of both endoprocts and ectoprocts, is the most comprehensive single treatise in the literature. Other important contributions have been made by: Schneider (1869), Claparède (1870), Nitsche (1870), Metschnikoff (1871), Allmann (1872), Salensky (1874), Schmidt (1876), Hatschek (1877), Joliet (1877), Repiachoff (1877), Barrois (1879; 1882; 1886), Hinks (1880), Harmer (1885; 1887; 1903; 1931), Ostroumoff (1885; 1886), Vigelius (1886), Pergens (1889), Prouho (1890; 1892), Seeliger (1890; 1906), Calvét (1900), Kupelwieser (1905), Marcus (1921; 1926a, b, c,), Waters (1925), O'Donoghue (1926), B. H. Grave (1930), and Rogick (1939).

Although a considerable amount of experimental work has been done during the past fifteen years on the behavior and metamorphosis of larval ascidians and a few

other sessile organisms, only a few observations have been recorded for the Bryozoa. The swimming movements of the larvae and their reactions to light have been described briefly in the earlier literature; but attempts to modify their behavior under controlled conditions have been confined largely to those of Marcus (1926a, c) and B. H. Grave (1930). The latter dealt exclusively with experimental modifications of the reactions of the larvae to light. The former, by employing vertical temperature gradients, demonstrated the existence of a fright-reaction and a lethal temperature zone at 38° C. for the larvae of the fresh-water species, *Plumatella fungosa*. He also briefly recorded the failure of these larvae to respond to an increasing oxygen-gradient and their lack of conformity to the generally accepted rules for determining the effects of viscosity and temperature on the speed of swimming movements.

McDougall (1943) discussed the breeding period, growth-rates and seasonal distribution of colonies of *Bugula neritina* in the Beaufort region and, after briefly referring to the behavior of the larvae, emphasized the need for further research. Since the embryology of this species has not yet been reported, a brief description of the larva, an account of the events that take place during metamorphosis and the duration of its various phases are presented. A review of the literature shows that the earlier embryologists only vaguely described the length of the post-fixational sequences of other species of Bryozoa. Among more recent writers, B. H. Grave (1930) has given an excellent description of the larvae of *B. flabellata* and *B. turrita* from the Woods Hole region and has recorded the growth rates during the later embryological stages, but his account of the early phases of metamorphosis is somewhat brief.

Distribution of Bugula Neritina

This semi-tropical species is abundant in the Dry Tortugas, Florida (Osburn, 1914), in Japan (Miyazaki, 1938), in Hawaii (Edmondson, 1944) and on the coast of California (Robertson, 1905). Canu and Bassler (1929) found it in the Philippines; Verrill and Smith (1874) have recorded it for the Bermuda Islands, Calvét (1900) for the southern coast of France and Osburn (1927) for Curaçao. Although it apparently does not range farther north on the Atlantic Coast than Beaufort, North Carolina, it is replaced along the northern half of the East Coast by a closely related species, *Bugula turrita,* which can be distinguished from it by the presence of avicularia.

The adult organisms are purplish-brown, branching colonies, which may reach a length of 10-12 cm. McDougall (1943) found that the vertical distribution of these colonies was most dense at a distance ranging from 6 inches to 3 feet below mean low water. Colonies near the surface were longer than those nearer the bottom and growth was more luxuriant. The distribution of adult colonies, therefore, differed considerably from that of the larvae. The writer found the bottom of a large raft to be the most suitable place for collecting adult specimens, which grew luxuriantly in close association with the ascidian, *Perophora viridis. Pennaria,* which is frequently associated with *B. neritina,* grew abundantly along the sides of the raft, but was almost entirely absent from the bottom. Light seems to be a controlling factor in causing the former organisms to attach and grow in deeply shaded regions and the latter in places exposed to bright sunlight. Along the western coast of North America, *B. neritina* becomes the dominant pile-dweller

during November, when it replaces the formerly predominant algal community, with which it is closely associated (Scheer, 1945).

Since *B. neritina* grew abundantly near the laboratory on rafts and pilings within arm's length of the surface, it was surprising to find a much altered situation less than a mile up the mouth of the Newport River. Here Bryozoa could not be found at all on pilings within three feet of the surface. Scrapings from regions lower down failed to bring up any specimens. A few sparsely growing colonies were found near the bottom of submerged objects that could be raised to the surface from a depth of 6 or 8 feet. Since samples of the water showed a surprising decline in salinity within a range of half a mile, it was considered probable that the salt content might be a controlling factor in bringing about the observed horizontal distribution in this region.

Materials and Methods

Since the histological details of the development of several species of Bryozoa are well-known, the writer used only living material for the embryological observations and the experimental work. Through the facilities offered by the U. S. Bureau of Fisheries, Beaufort, North Carolina, the larvae for these studies were obtained from sexually mature colonies placed in finger-bowls and kept in running water. These colonies usually yielded a considerable number of larvae each day for a period of ten days to two weeks. In some instances, however, larvae were produced in enormous numbers for five or six days; but at the end of that time they were no longer shed, and the colonies had to be replaced with fresh ones. During the summers of both 1944 and 1945 it was observed that there were periods lasting about a week when larvae were almost unobtainable in large numbers, even when fresh colonies had been gathered the night before. These periods occurred at approximately the same time each year, towards the end of the first wek in August. Since there seemed to be no unusual variations in temperature or sunshine, the cause of this phenomenon was not determined.

Although it would be preferable, indeed, to use only larvae from a single colony for each set of experiments, nevertheless the small number that would be obtainable by such a procedure practically precludes the attainment of such a desideratum. Some of the variations in the behavior of the larvae under similar experimental conditions may be attributable, perhaps, to differences in genetic strains. It seems more likely, however, that the length of time that the larvae have remained in the ovicells before being shed is a more important factor in introducing seemingly fortuitous variations in the length of the free-swimming period. The larvae, as a rule, show a remarkable uniformity in their behavior under similar environmental conditions; genetic variations seem to play only a minor role in determining the length of the free-swimming period.

For observations on the responses of the larvae to light and gravity at reduced temperatures, stender dishes 5 cm. in diameter and 3 cm. deep were used exclusively during the first summer. During the second summer, homeopathic vials 8 cm. long and 1.5 cm. in diameter were used for these observations and for those on the effects of salinity. For most of the light experiments, vials 16 cm. long and 1.8 cm. in diameter were employed. These were scrubbed carefully with soap and water and then rinsed thoroughly with sea water. A light-box, $8 \times 8 \times 30$ cm., was

constructed so that light could enter only one end of a vial placed within it. Well-slides were used for microscopic work.

Shortly before the experiment was to begin, finger-bowls containing adult colonies were placed near a window that admitted diffuse daylight to the laboratory. When larvae were liberated, they swam immediately towards the side of the dish nearer the window and collected there in swarms just beneath the surface film. They could then be pipetted to the various experimental vials, which were numbered so that a record of the time of placement could be kept. Since the liberation of larvae is photoperiodic, generally beginning 30–40 minutes after the parent colonies are exposed to light, active specimens could be obtained throughout the day, if the containers were placed in a darkroom on the preceding evening and removed to a window a short time before the larvae were needed. Although active swimmers could be obtained as late as 3:30 P.M. (E.S.T.) by this method, the number of larvae shed in the afternoon was generally small in comparison with the number liberated between 7 A.M. and noon. Since the darkroom was not equipped with a system of running sea water, the food supply of the adult colonies may have been inadequate under these conditions for the proper development or liberation of larvae. The finger-bowls were generally kept in the aquarium, which was situated in a fairly well-shaded spot; only on days when the room was flooded with light from an early hour were the larvae shed in large numbers before the experiment began. Observations were usually made between 7A.M. and noon, although in some cases they had to be carried out until 6 P.M. and in one case until 10 P.M. Larvae were most abundant during the first two hours after exposure of the parental colonies to light.

Observational Section

Structure of the larva

The larva of *B. neritina* resembles other larvae of the same genus in its structural features and its general contour. It may be described as pyriform rather than peach-shaped, however, with average dimensions 0.20–0.22 × 0.27–0.30 mm., although smaller specimens were observed at times. At the narrower end of the body the convex apical organ, the "calotte" of Barrois (1879), is clearly set off from the rest of the larva by a crown of rigid cilia and by a circular groove or collarette, the pallial furrow (Fig. 1). Its coloration is somewhat lighter than the rest of the larva, which looks green by reflected light, but brown by transmitted light because of the numerous pigment granules imbedded in the body. The middle of the apical organ has a darkly pigmented spot whose center shows a tiny clear area that sometimes disappears when the surrounding tissue suddenly contracts (Fig. 4). This spot marks an opening into a shallow cavity in the interior of the larva. The broader end, which corresponds to the oral depression of larvae that have a rudimentary or functional alimentary canal, contains in its center an invagination forming the relatively voluminous internal sac or sucker, the "saugnapf" of Pergens (1889), whose opening to the outside is marked by a ring of black pigment (Fig. 3). The internal sac becomes so distended by a secretion of granular material from its walls that the whole region between the equator and the oral depression bulges like the broader end of a pear. The median furrow, which B. H. Grave (1930) called a lateral groove, is clearly defined by its lighter coloration and by the absence of pigment (Fig. 2). In the median furrow near the equator is a tuft of four, long,

blunt vibratile flagella that beat in unison. This is the "plumet ciliaire" of Barrois (1877). The median furrow and its associated glandular structures constitute the piriform organ. In some specimens there are two, small, darkly pigmented eye-spots near the tuft of flagella and symmetrically placed with reference to the median furrow. They can be seen from the opposite side through the somewhat transparent larva (Fig. 1). On the side of the larva opposite the median furrow are two, prominent, black, diamond-shaped eye-spots lying almost on the equator and about 90° apart. These structures are a constant feature of the larvae and serve as excellent landmarks for their proper orientation during swimming movements. A tiny, white area that appears frequently in the center of each eye-spot, when light falls at the proper angle, is somewhat difficult to interpret; probably it is caused by a concentration of light brought about by a crystalline lens that covers the eye-spots, as Nitsche (1870) has described for another species of *Bugula*. The locomotor cilia, which cover the body except in the region of the apical organ, are more active near the apical organ than on the half of the body containing the oral depression; if stationary larvae are observed, water currents can be seen flowing from the region near the apical organ to the equator and then outwards and back again to complete a circle. Since locomotor cilia of most bryozoan larvae are found only on the enlarged coronal cells that form an elevated ring around the body of the organism, Barrois (1877) has interpreted the presence of cilia on the whole body of *Bugula* larvae as an indication that the corona has spread out enormously in these forms, so as to occupy the whole region between the apical organ and the oral depression. This view is supported by the fact that the corona of *Flustrella hispida* is a single band around the oral pole, whereas it is separated into two distinct bands in the larva of *Cyphonautes compressus* and in other forms having a similar type of larva. It is quite possible, however, to regard the condition in *Bugula* as primitive; condensation of the cilia into bands may not have occurred until a later period of phylogenetic development.

Changes in body contour, accompanied by elongation of the larvae, can be observed frequently. In some cases larvae look lobular rather than pear-shaped, as though they were divided into four lobes by two deep constrictions running at right angles to each other (Fig. 5). This appearance results from a contraction in the region of the equator accompanied by a depression of the apical organ.

Orientation and swimming movements of the larva

The larvae usually swim with their long axes tilted at an angle of 45° with the vertical, if they are advancing in a definite direction and are not in contact with the bottom of the container or the surface film. Both the apical organ and the tuft of vibratile flagella are in advance and the median furrow is directed downward. In this respect the larvae differ from those of *B. flabellata,* which swim with their long axes directed horizontally, and from the larvae of the Escarina, in which the long axes are vertical (Barrois, 1877; 1886). Spiral movements, however, can be observed frequently. While spiralling, the organism usually has its median furrow on the outside of the spiral. This is probably why B. H. Grave (1930) called it a lateral groove. (He clearly described the function of the flagella and cilia in these movements.) If the larvae are in contact with parts of the container or the surface film, they progress by creeping movements with the median furrow and the vibratile flagella in contact with the surface. Sometimes, while on the bottom, they spin on

their long axes and show in quick succession first the median furrow and then the pigmented eye-spots.

Observations of these diverse methods of locomotion have brought about a certain amount of confusion in the literature on closely related species. Calvét (1900), for instance, in referring to the larva of *B. sabbatiere* described the half containing the eye-spots as posterior and the other half bearing the median furrow as anterior. B. H. Grave (1930), on the other hand, referred to the apical organ of *B. flabellata* as the anterior end. His orientation is in agreement with that of Barrois (1877). As Grave (1930) noted, it is necessary to distinguish the anterior half of the larva, as determined by its forward motion, from the morphological anterior end; for the mouth, when one is present in bryozoan larvae, is opposite the apical organ. Barrois (1877) cited similar cases showing the different interpretations of various observers regarding the orientation of the cyphonautes larva of *Membranipora pilosa* (*Electra pilosa*) (p. 232). It seems worth noting that the sensory structures, the apical and piriform organs, are in advance as the larva moves forward and first come in contact with the environment. According to Prouho (1890) the apical organ of *Flustrella hispida* is connected to the piriform organ and also to the cells of the corona by nervous tissue. There can no longer be any doubt about the sensory nature of either the apical or piriform organs.

During the early part of the larval period, relatively long distances are covered by a spiralling motion. Finally there is a slowing down of movement, accompanied by a change in the method of progression. Spiralling ceases, and the larvae now swim in circular planes parallel to the substrate; the radii of the concentric circles formed by the larvae decrease in length as the onset of metamorphosis approaches. Eventually they slow down enough to be kept within the low-power field of the microscope. While swimming in circles, larvae often behaved as if a very fine, sticky thread trailed beneath the piriform organ, which was downward at the time. Although the thread itself could not be seen, several larvae were observed to pull debris, with considerable difficulty, at a distance of several millimeters; they could be pushed and pulled with a probe at a short distance from their bodies.

Before fixation, larvae alight on a suitable substrate and begin to rotate counterclockwise on an axis running from a point midway between the eye-spots to the opposite side, where the piriform organ touches the substrate. This continues for 5

PLATE I

FIGURE 1. Profile view of the larva showing pigmented eye-spots. 1. Convex apical organ, 2. Crown of rigid cilia, 3. Collarette or pallial furrow, 4. Eye-spots on the opposite side, seen through the somewhat transparent larva, 5. Ciliated corona, 6. Eye-spot, 7. Pigmented band.

FIGURE 2. Profile view showing the median furrow and associated structures (the piriform organ). 1. Part of the glandular system, 2. Eye-spot, 3. Tuft of vibratile flagella, 4. Median furrow, 5. Oral depression.

FIGURE 3. A three-quarters view showing the position of the long axis during spiral movements. 1. Opening to the internal sac, 2. Oral depression.

FIGURE 4. View showing the apical organ. 1. Opening in the apical organ, 2. Crown of rigid cilia, 3. Pallial furrow.

FIGURE 5. View showing the larva compressed in the region of the equator with the apical organ retracted. 1. Retracted apical organ.

FIGURE 6. Profile view, 15 seconds after fixation, showing characteristic hour-glass appearance. 1. Pallial furrow, 2. Vibratile flagella, 3. Ejected holdfast.

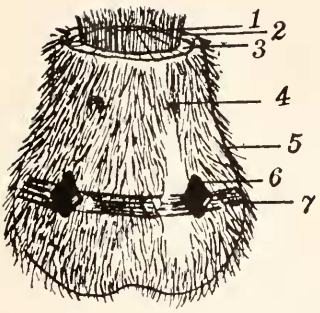


Fig. 1

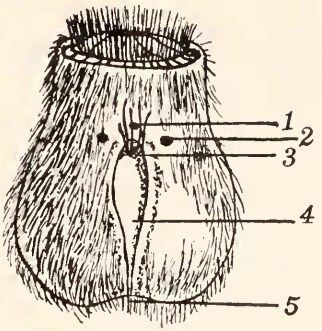


Fig. 2

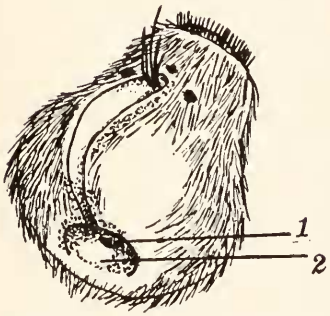


Fig. 3

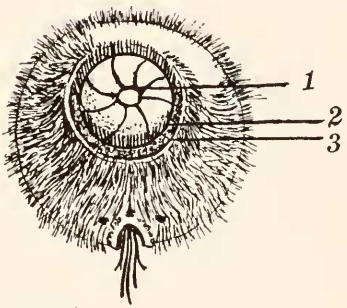


Fig. 4

Fig. 5

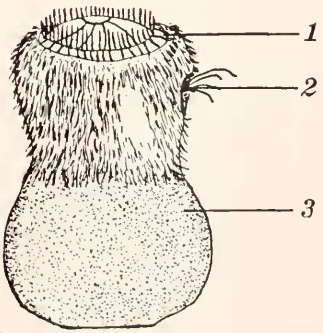


Fig. 6

PLATE I

or 10 minutes. Rotation gradually decreases in speed and finally stops just before attachment. The flagella of the vibratile plume enable the larvae to choose places suitable for fixation. Light and shadow undoubtedly play an important role in the selection of a favorable spot.

Metamorphosis

Just before fixation the larva actually appears to be anchored to the point of attachment by an invisible thread-like substance. If it is going to attach to the bottom of a well-slide, it alights with the eye-spots uppermost and the median furrow along the substrate. While it revolves slowly, always in a counterclockwise direction, it changes its shape, so that it looks like an oblate spheroid, bulging at the equator and flattened in the region of the apical organ and oral depression. Perhaps these changes in body contour indicate the establishment of a new polarity just before fixation, since Child (1925) found that a reversal of polarity occurs in hydrozoan larvae immediately before attachment.

Before setting, the vibratile flagella are very active, feeling the surface of attachment, while the larva revolves slowly for 3 or 4 minutes. At the moment of fixation the median furrow grasps the substrate by means of its ridges, which are both muscular and glandular. Within a second or two the larva elongates, so as to double the length of the long axis of its body. Then very suddenly the internal sac, containing a white, translucent, slightly granular, jelly-like material, is everted and forms a light colored, rounded mass beneath the organism, which is almost as large as the larva itself.

Simultaneously with the eversion of the internal sac, the median furrow releases its grip on the substrate and the larva rotates, so as to change the original long axis of the organism from a horizontal to a nearly vertical position. The apical organ, therefore, is brought upwards, but in such a manner that it faces away from the source of illumination at an angle of 15 or 20° from the vertical. As the larva rotates, it orients itself by squirming movements, until the eye-spots are located on the lighted side. The writer did not observe that the apical organ in this species points towards the source of illumination at the time when metamorphosis commences, as Grave (1930) has described for *B. flabellata*. After fixation the aboral

---

PLATE II

FIGURE 7. "Umbrella stage" (semi-diagrammatic optical section). 1. Epithelium from the pallial furrow, 2. Holdfast.

FIGURE 8. Diagram showing epithelium from the pallial furrow forming a firm union with the holdfast. 1. Epithelium from the pallial furrow, 2. Reflected original larval covering.

FIGURE 9. The fixed larva viewed from above, 13 minutes after fixation. 1. Ringed cavity surrounded by degenerating ciliated larval covering (the corona), 2. Ciliated covering in the process of degeneration, 3. Transparent anhiste zone (the holdfast), 4. Eye-spots seen through the new larval covering (epithelium from the pallial furrow).

FIGURE 10. The fixed larva, viewed from above, 18 minutes after fixation. The horse-shoe shaped mass has increased in diameter.

FIGURE 11. The cystide, nine hours after fixation, showing a cut in the region where the piriform organ was situated. The rudiment of the polypide can be seen in the center.

FIGURE 12. The cystide somewhat older than that shown in Figure 11 (about 12 hours after fixation). The rudiment of the polypide is already well-formed. 1. Granular material being extruded into the surrounding water, 2. Break in the outer cystide wall, 3. Rudiment of the polypide.

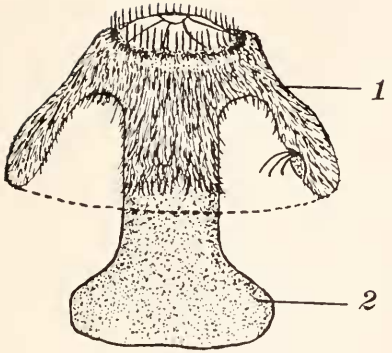


Fig. 7

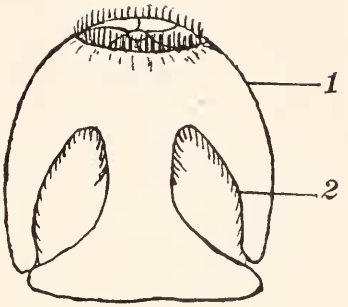


Fig. 8
(diagrammatic)

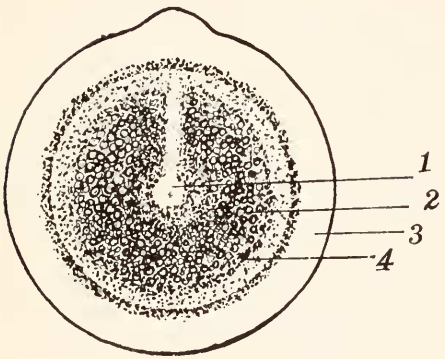


Fig. 9

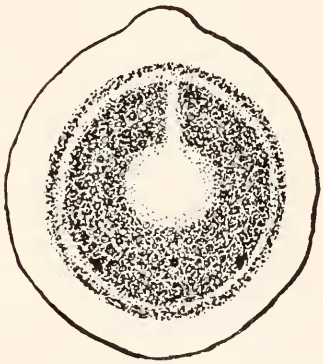


Fig. 10

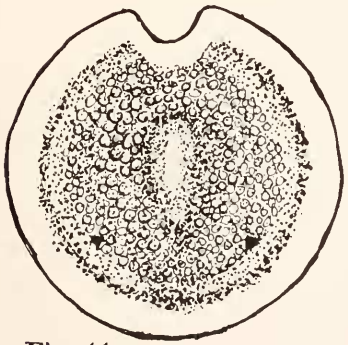


Fig. 11

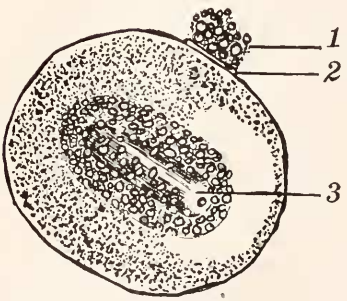


Fig. 12

PLATE II

end of the attached larva and later the apical part of the first zoöid are oriented so as to point away from the direction of the incoming light.

With the eversion of the internal sac, which formerly distended the oral hemisphere of the unmetamorphosed larva, the central portion of the body collapses somewhat and forms a narrow column supporting the apical organ on top and connecting it to the upper part of the evaginated internal sac beneath. Thus the central region of the organism becomes constricted and the larva assumes a characteristic hour-glass appearance (Fig. 6). A cavity is formed in the region formerly occupied by the internal sac. This hour-glass appearance lasts only 3 or 4 minutes, while the larva orients itself to light. If the slide is subsequently turned through an angle of 180°, no further orientation will take place; the eye-spots will remain on the side opposite the source of illumination.

Then very suddenly the apical epithelium unfolds from the pallial furrow, where it was tucked-in to form the collarette, and extends outward and downward, carrying with it the upper border of the ciliated larval covering as it migrates towards the adhesive plate (Fig. 7). This is the "umbrella stage" of Barrois (1886). The ciliated covering of the larva thus becomes reflected outward and downward, until it finally becomes completely enclosed by the apical epithelium (Fig. 8). The cells of the latter become stretched and flattened, so that their resultant transparency allows prominent landmarks like the pigmented eye-spots to be seen beneath it. The latter are carried downward during this process, until they finally rest just above the everted internal sac, where they form prominent bulges; they remain visible for 15 or 20 minutes after fixation (Fig. 9). Then with a sudden contraction the apical organ is pulled down towards the everted internal sac, which becomes flattened to form an adhesive plate. The larva now looks like a rounded cup inverted over a plate.

Just after the aboral organ is pulled down towards the adhesive plate, the fixation substance seems to grow larger in diameter, when viewed from the apical pole. This is probably caused by a flattening-out of the adhesive material and by a simultaneous constriction of the central, ciliated, papilla-like structure, which forms a ring of small diameter. At this time the opaque parts of the organism, the ciliated former outer covering and the apical organ, measure 0.14–0.15 mm. in diameter. Then the size of the adhesive plate seems to decrease again and at the same time the central ciliated mass widens, so that the clear area in the center becomes larger (Fig. 10). This appearance of a decrease in the diameter of the adhesive material can be attributed to the beginning of a union of its outer margin with the lower border of the reflected epithelium from the pallial furrow, which forms a new covering for the organism. The adhesive apparatus itself consists of a lower layer of transparent material, probably a cement-like adhesive substance formerly contained in the internal sac, and a smaller layer on top; the latter, which is somewhat pigmented, is the border of the internal sac.

The lower border of the epithelium from the pallial furrow does not form a firm union with the adhesive plate until 20 or 30 minutes after fixation. The cilia, at the margin of the former larval covering and the epithelium from the pallial furrow which covers it, may be seen beating for as long as 10 or 15 minutes after attachment. The vibratile flagella continue to move rapidly at first and then spasmodically for 5 or 10 minutes longer, until the cells to which they are attached have been carried well into the interior. Before these cells have migrated to a position nearer

the center of the larva, the flagella can be seen sticking out on top of the adhesive plate, which forms a prominent bulge in this region (Fig. 9). In many instances granular material was observed to be extruded into the surrounding water, as if the adhesive plate were ruptured by the movement of the flagella. Finally, they retreat completely within the new outer covering.

As a result of all these changes, the vibratile flagella and the associated piriform organ, as well as the whole ciliated original larval covering, have become enclosed by a new outer covering formed by epithelium from the pallial furrow. At this time the larva looks like a sac, closed on the bottom by the adhesive plate; its top and sides are formed by the apical organ and by the epithelium from the pallial furrow respectively. Its wall is lined on the inside by the ciliated former larval covering, which looks like a C-shaped mass, a ringed hole, within the larva. This C-shaped appearance can be attributed to the fact that the ciliated epithelium is darkly pigmented and opaque, except in the region of the piriform organ. What looks like an opening in the ring is undoubtedly the unpigmented piriform region, for the flagella always retreat inward from this side of the larva. After 10 minutes this ring gradually widens, the central clear area grows larger in diameter and the pigment seems to diffuse. This change probably results from the disintegration of the ciliated larval covering and of all the organs that served a useful purpose in the free-swimming organism.

Shortly after the apical epithelium emerges from the pallial furrow to form a fold which advances downward towards the adhesive plate, the apical organ loses its vesicular appearance and the tiny opening in its center begins to grow wider. During this time the apical epithelium can be seen advancing outward very slowly, as if it were being pulled and stretched by the new larval covering. This partially clarifies the observation that the upper margin of the old ciliated covering retreats inward very gradually, as the amount of material of the new covering is augmented very slowly by additions from the apical region.

Apparently the whole apical organ invaginates into the center of the C-shaped depression and undergoes histolysis, just as Prouho (1890) described for *Flustrella hispida.* He stated that the rudiment of the polypide, although it makes its appearance in this region later on, does not develop from the invaginated apical organ but rather from a meso-ectodermal disc formed independently from apical epithelium and from an internal mesodermal rudiment. The apical organ, like all the other organs that functioned in the free-swimming larva, degenerates after invagination.

Within an hour after fixation the adhesive plate, in the region where the vibratile flagella retreated, becomes deeply constricted by a notch, which can be seen for 4 or 5 hours after attachment (Fig. 11). One or two dark protrusions sometimes appear on the side opposite the notch. These are apparently the eye-spots pressing against the outer covering of the metamorphosing organism.

The larva passes into a stage known as the cystide about an hour after fixation. During this time the ectodermal walls secrete a thick covering, the ectocyst, which makes observation difficult. The further development is essentially similar to that described by Prouho (1890) for *Flustrella hispida.*

Eight or nine hours after fixation the rudiments of the polypide can be distinguished near the top of the elongating mass opposite the basal end and in the center of the now poorly-delimited C-shaped ring (Fig. 11). Twenty-four hours after fixation the zoöecium and the polypide within it are clearly recognizable. The

zoöecium itself is club-shaped, broader at the apical end where the lophophore will appear, and gradually narrowing towards the basal end, but flaring-out again at the point of attachment. At the end of 24 hours the young zoöids measure 0.6–1.0 mm. By 48 hours they have reached a length of 0.74–1.2 mm. and at 49 hours, 0.8–1.3 mm. The lower four-fifths of the body of the 24-hour zoöid is composed of transparent material derived from the adhesive plate; within it the tube-like polypide can be seen extending to the base of the holdfast. The apical end is made up of pigmented material, which forms a somewhat elongated, spherical mass that looks considerably like the unmetamorphosed larva. The transparent zoöecial wall encloses this spherical mass. At the extreme tip of the apical end two black spots mark the lophophores of the first and second individuals of the colony. The tentacles of the first are usually everted at the end of 28 hours; by this time a bud for the second individual is already well formed. Its tentacles are not everted, however, until approximately 48 hours have elapsed.

Since the lophophore and mouth of the first individual of the colony are formed in the region formerly occupied by the apical organ of the larva, there is a reversal of polarity through 180°. The oral end of the larva becomes the aboral end of the ancestrula of the colony.

The eversion of the internal sac and the events that take place during the "umbrella stage" occur so rapidly that the writer had to observe 40 or 50 larvae in order to get a clear grasp of the details. The "umbrella stage" can be seen best, when larvae attach to the lateral walls of a well-slide. On the other hand, the fimbriated nature of the holdfast can be seen only when larvae attach to the surface film. In this case the piriform organ is upward and the eye-spots are downward at the moment of fixation. Then the internal sac is suddenly everted upwards above the larva like a relatively huge balloon. The larva then turns, so as to bring the apical organ downward beneath the surface and the oral depression upward.

Larvae that attach to the surface film show no irregularities of development. The tentacles of the first individual and sometimes the second are everted in a normal manner, and they snap vigorously during active feeding. They are quickly withdrawn into the tentacular sheath, if the water is disturbed by jarring the table. When they are touched with a probe, their holdfasts cling tenaciously to it. Although the longevity of these larvae was not determined, on several occasions they were still alive 3 or 4 days after fixation. By this time the young zoöids had turned over, so that the tentacles protruded upwards. Strangely enough, they formed a continuous chain by means of thread-like projections that united each of them to the adjacent zoöids.

Although Visscher (1927) found that more bryozoan larvae affixed themselves to red test panels than to green, black or yellow ones and that none attached to white panels, the writer found no evidence that the larvae of *B. neritina* showed any preferences for pieces of red brick placed within the finger-bowls. They did prefer, however, the rougher surfaces of submerged test panels to the smoother parts, just as Visscher found. It appears likely that these larvae prefer to attach to organic material. In nature they frequently affix themselves to algae and to established bryozoan colonies. Although the presence of bacterial slime favors the attachment of most macroscopic organisms (ZoBell and Allen, 1935) and seems to be a prerequisite for the fixation of barnacles (Hillen, 1923), it does not have

any influence on bryozoan larvae (Scheer, 1945; Miller, 1946). Scheer (1945) found that bryozoan larvae would not attach to glass plates unless they had been submerged for at least two weeks; but they were influenced only by the abundant diatom population that had accumulated. The writer observed, during a three-week period in which larvae were shed daily in a finger-bowl, that 20 or 30 had attached to the body of a young *Styela* that was 2 or 3 cm. below the surface of the water, but none attached to the side-walls at this level.

Experimental Section

The effects of temperature on the responses of the larvae to light and gravity

Grave (1930) found that the larvae of *Bugula flabellata* are photopositive during the greater part of their free-swimming period but become negatively phototactic shortly before fixation.[1] Mast (1911), too, found that other organisms show a similar reversal of phototropism from positive to negative as they approach the end of the pelagic stage. The larvae of *B. neritina*, however, under ordinary laboratory conditions of temperature and salinity were never observed to become negatively phototactic at any time; swarms of metamorphosed larvae could always be found on the lighted side of the container within an hour after they had been shed. Furthermore, they maintained a definitely negative geotaxis throughout the entire larval period and attached in large numbers just beneath the surface film. In some instances they attached to the sides of the container, but fixation always took place at or near the surface. Here, then, was a paradox! What factors in nature, not operative under the unusual conditions of the laboratory, could be responsible for causing larvae to attach near the bottom of the channel and in regions not exposed to the direct rays of the sun? At first, temperature seemed to be the most likely factor, for setting of the larvae occurs most abundantly during April and May (McDougall, 1943), when the sea water is relatively cooler than it is during the summer months. Again, it seemed likely that the more normal behavior of the Woods Hole species in the laboratory might be due not so much to specific differences as to lowered temperature, for the average temperature prevailing there during May, June, July, and August is 6° below that at Beaufort.

The hypothesis that temperature might be a controlling factor in bringing about the observed distribution was first tested during the summer of 1944. Although a reduction of temperature to coincide with that of the channel during April and May did not change the geotropism of the larvae, further cooling had a marked effect. The results of two experiments, selected from many similar ones with both stender dishes and vials, are summarized in Table I, which shows the responses of the larvae to both a descending and an ascending gradient. Counts were made at approximately 2.5–3.0-minute intervals during a 30-minute period, just long enough to reverse the geotropism of all the larvae. The vials were immersed in ice-water, and temperatures were taken at the bottom, where the water was 4 or 5° cooler than on top. Although no definite temperature could be determined as the critical one at which all the larvae were either geopositive or geonegative, nevertheless the data in this table and in others not recorded show that the greatest shift

[1] The terms, phototropism and phototaxis, will be used synonymously in this paper, without regard for their original meaning as described by Mast (1911, p. 253). These organisms may be either phototactic or photopathic, according to the older terminology.

from one response to the other occurred between 20 and 23.5° C. It can be noted that larvae exhibit a kind of inertia to a change of position, when either ascending or descending temperature gradients are employed. A change in geotropism is a function of time as well as of temperature. Thus, a drop of 13° had effected a change in only 37 per cent of the larvae; the remaining 63 per cent were affected by a change of only 3°. During the last 15 minutes of the experiment when the temperature remained constant (at 7°), 35 per cent of the larvae descended to the bottom. Similarly 65 per cent had not responded to a rise of temperature from

TABLE I

Temperature	Number of geopositive larvae	Percentage geopositive
23.0	0	0
14.1	16	34
10.0	17	37
9.5	19	41
9.0	22	48
8.5	24	52
7.5	25	54
7.0	30	65
7.0	33	71
7.0	34	74
7.0	36	79
7.0	39	85
7.0	46	100

Temperature	Number of geopositive larvae	Percentage geopositive
7.0	46	100
10.0	43	93
12.0	40	87
14.0	40	87
16.0	34	73
18.0	30	65
20.0	21	45
20.5	15	33
21.5	12	26
22.0	3 (active)	6.5

7 to 18°. During a subsequent 15-minute period, however, all but 6.5 per cent had reversed their positions in the vial, even though the temperature rose only 4°. Nine larvae, having attached at the bottom, could not respond.

Not only does a reduction of temperature change the geotropism of the larvae, but it also prolongs the free-swimming period. When normal sea water was cooled to 7 or 8° C., more than 80 per cent of the larvae were found to be active at the end of an hour, in contrast to only 6.7 per cent in the controls. In general, it may be stated that the average free-swimming period can be prolonged 2–3 hours by cooling the medium. Although the extreme limit to which the natatory period could be extended by treatment with cold water was not determined, nevertheless, in several instances 20–30 per cent were found to be active at the end of 5.5 hours. If we take into consideration the fact that the majority of larvae metamorphose at room temperature within 30–60 minutes after release from the ovicells, the effects of tem-

perature on the length of the swimming period are very marked. Marcus (1926c) made similar observations on the larvae of fresh-water bryozoans.

The motility of larvae can also be modified by temperature. When quiescent larvae, which had not yet metamorphosed, were taken from containers at room temperature (28° C.) and placed in water at 7–18°, they immediately became active. Although it is difficult to judge the degrees of motility merely by observation, apparently the greatest amount of activity was exhibited when the temperature was reduced to 16°. At 12° it was still considerable, but less than at 16°. At 7° motility was still more reduced and activity was largely confined to creeping movements.

Some organisms reverse their reactions to light when the temperature is reduced. Thus, *Euglena* (Mast, 1911), *Chromulina* (Massart, 1888), *Acartia* (Esterly, 1919) and haematococcus swarm spores (Strasburger, 1878) are photopositive at room temperature (18–20° C.) but become negative when the temperature is reduced (to 4–8° C.) Others, such as *Polygordius* (Loeb, 1905), change from positive to

TABLE II

Temperature	Percentage nearer the source of illumination	Percentage intermediate	Percentage farther from the source of illumination
22	35.0	31.2	33.8
20	73.0	4.0	23.0
19	19.4	16.0	65.0
19	31.0	14.0	55.0
19	81.0	0	19.0
17	76.2	14.3	9.5
13	80.0	20.0	0
9	80.0	0	20.0
7.5	0	51.7	48.3

negative with a reduction in temperature. *Daphnia,* on the other hand, shows no reversal of phototropism when the temperature is changed (Yerkes, 1900). In order to determine the effects of various temperatures on the phototropic reactions of the larvae of *B. neritina,* the following observations were made (Table II). Contrary to expectations based on casual observations, these experiments led to the conclusion that in cold water the larvae merely lose their otherwise intense positive reaction to diffuse daylight and become more or less indifferent, swimming back and forth towards and away from a source of illumination. Although B. H. Grave (1930) had observed that this activity precedes a definitely negative reaction in *B. flabellata,* the writer found that setting of the larvae of *B. neritina* occurred more or less at random in water at reduced temperatures.

Since larvae metamorphosed, apparently without any exceptions, on the lighted side of finger-bowls containing water at room temperature, for a time it was considered that they were photopositive at the time of attachment. When small vials were used, however, fixation occurred in 70 per cent of the cases either at random or at the center of the vials in circular masses just under the surface film. Approximately 50 per cent were on the half of the disc farther from the source of illumination. These settings seem to indicate that even at room temperature larvae become indifferent to light shortly before fixation. Since phototropism is correlated

with the amount of activity of the larvae, active swimmers are almost universally photopositive; as activity decreases, they lose their intense positive reaction to light and become more or less indifferent. Because a reduction of activity occurs only a very short time before metamorphosis, larvae under laboratory conditions usually cannot move very far from the lighted side of the container before fixation takes place. Even when larvae are made to react negatively to light by experimental prolongation of the free-swimming period, as described later, this reaction is never so intense as the positive one. Perhaps this observation explains why McDougall (1943) found the highest incidence of settings not in the most dimly illuminated part of the light box, but in the adjacent chamber where slightly more light was admitted.

Phototropism vs. geotropism

Marcus (1926a) stated that the negative phototropism exhibited by the larvae of the phylactolaematous bryozoans shortly before fixation dominates a coexistent negative geotaxis and forces them to seek places of attachment at some distance beneath the surface of the water.

Since in nature both light and gravity act in approximately the same vertical plane, it might seem logical to identify a positive geotaxis with a negative reaction to light. The behavior of the larvae of *B. neritina* at reduced temperatures, however, demonstrates that they may become geopositive without showing a simultaneous negative reaction to light. The following experiments were set up to determine which of the two tropisms is dominant in this species.

TABLE III

No. of trials	12
Average temperature (mean)	28.1 $\sigma = \pm 1.1$ (25–29 degrees)
No. of larvae	332
Percentage geopositive	28
Percentage intermediate	14.2
Percentage geonegative	57.8
Average time of exposure in minutes	8.9 $\sigma = \pm 1.8$ (8 to 10 min.)

If the response of the larvae to light were dominant to their geotaxis under given conditions, they ought to swim against a gravity gradient towards or away from a source of illumination, depending on their phototropism at the time. Consequently, on several different days larvae were made geopositive by treatment with low temperatures. Vials 16 cm. long and 1.8 cm. in diameter were used; some of them were cooled to 5° C., some to 6°, and others to 8°. They were then placed inside the light box, so that rays from a 100-watt bulb could enter only from the top. The source of light was 22 cm. from the bottom of the vials, where the larvae were swimming. As might be expected from the previous discussion of the indifference towards light exhibited by larvae at reduced temperatures, a positive phototropism under these conditions could not be demonstrated. Only 2.1 per cent of the larvae in 4 of the vials swam to the top; in the other three, none changed their positions as the result of the illumination. Six trials were then made with the light-box arranged so that rays could enter only from below. The light source was somewhat nearer the larvae in these cases, being only 6 cm. from the bottom of the vials. In all cases only negative results were obtained. The larvae did not swim away

from the bottom in a negative response to light. These experiments indicate, then, that larvae at low temperatures are either indifferent to light or that their positive geotaxis under these conditions is strong enough to counteract any reaction to light.

Different results were obtained with larvae kept at room temperature. When pipetted to vials, they were geonegative at the beginning of the experiment, and the vials were then placed in the light-box so that they were illuminated only from below. Many of the larvae swam downward. Counting was done immediately after the vials were removed and the results of 12 trials are recorded in Table III. Larvae were always observed to swim upwards, when the vials were removed from the source of light, in all the trials except one; they were never seen swimming in the opposite direction. Of the 332 larvae tested only 57.8 per cent remained geonegative after treatment. In the control vials, however, 91.2 were negatively geotactic. If due allowance is made for the probability that some of the larvae in the experimental vials had metamorphosed during treatment and could not respond, the contrast in percentages would indicate that at room temperature a positive phototropism is dominant to a negative geotaxis in approximately half the larvae. On the other hand, these experiments show that larvae respond positively to light with much greater readiness when they can swim along a horizontal plane than when they must swim downward against what appears to be a buoyancy gradient. In one of the vials illuminated from below all the larvae became geopositive and metamorphosed on the bottom, but, since this vial was exposed to alternate periods of intense and diffuse light, it seems likely that a positive geotaxis resulted from the prolonged activity induced by alternate periods of light. Subsequent experiments demonstrated that any condition that prolongs the activity of the larvae eventually induces a positive geotaxis.

The effects of light intensity and darkness

Changing the intensity. A rheostat was used to govern the intensity of a 100-watt bulb placed 8 cm. above a vial enclosed by the light-box. A ten-minute exposure to dim light was followed by a ten-minute exposure to intense light. Although 15 trials were made, all attempts to bring about a downward migration of the larvae by suddenly either increasing or decreasing the intensity of illumination gave no results. Apparently the larvae of *B. neritina* are not affected by changes of light intensity, as so many plankton organisms are.

Intense light. Since strong illumination often causes a reaction in many animals which is opposite to their behavior in diffuse light, experiments were performed by using both artificial illumination and direct sunlight. The artificial illumination was at least three times as intense as that employed in the experiments described above or those summarized in Table III. Sixteen-cm. vials of normal sea water were placed 3 cm. below and at the side of a 100-watt bulb, and a water jacket was used to maintain a constant temperature of 29° C. At the beginning of the experiment larvae removed to these vials were very active, intensely photopositive and were swimming on top. By the end of 30 minutes, however, 41.8 per cent of the larvae in the experimental vials had descended to the bottom, whereas only 9.8 per cent became geopositive in the controls. Apparently a fairly high percentage of larvae at room temperature is negatively phototropic to intense light. The data obtained with strong sunlight, although comparable, were not clear-cut enough to

warrant publication, since temperature may have modified the results somewhat. Nevertheless, there is some evidence that strong sunlight causes larvae to move downward from the surface to regions of lower light intensity

Both strong sunlight and artificial illumination induce larvae to move along a horizontal plane away from the side where the rays enter the medium obliquely. Although strongly photopositive at first, by the end of 20 minutes all the larvae (in three vials) had moved away from the bulb to the distal side. Those exposed to sunlight remained photopositive much longer; but by 1.5 hours all had become photonegative in two of the vials and only 5 per cent remained photopositive in the third. In the latter, 45 per cent were intermediate and 50 per cent showed a negative reaction. Here again there is evidence that light is more effective in causing larvae to move along a horizontal plane than up or down, either towards or away from a source of illumination.

The negative reaction of larvae to intense light would seem to be significant. Even a casual observation of the distribution of various species of *Bugula* near the water line on piers and docks will show a preponderance of colonies on the north side of objects and in shaded places. The evident fact that in nature the majority of larvae are negatively phototropic at the time of attachment may be explained on the basis that they swim away from regions receiving the direct rays of the sun after a certain amount of exposure to intense light, a behavior that they do not show in a laboratory illuminated only by diffuse light. The opinion of Esterly (1919) that the physiological state of animals is changed, when they are removed from the ocean to the laboratory, would seem to be true only if all the conditions prevailing in their former surroundings are not exactly duplicated. Since the light-box used by McDougall (1943) was placed only 6 inches below low water mark, the amount of absorption of the sun's rays was probably insignificant; hence it should be expected that the larvae would be photonegative as described. Whether larvae far beneath the surface at the time of attachment show a negative phototaxis like those near the water-line can only be conjectured. Since absorption would considerably reduce the intensity of sunlight received by the lower regions, it might be expected that they would be either photopositive or indifferent, like those in the laboratory under conditions of diffuse daylight.

Darkness. Darkness sometimes inhibits the movements of animals and may change their reactions to both light and gravity. The work of Holmes (1905) on *Ranatra* led him to conclude, "The causes that produce the negative reaction [to light] are, as a rule, those which lead to diminished activity and excitement. Cold, exposure to darkness, and the quieting effect of contact stimuli lead to a condition of lessened excitability and, perhaps as a result of this, to a negative reaction to light" (p. 317). Mast (1911) obtained a reversal of phototropism in the larvae of *Arenicola* by subjecting them to a variety of agents, such as diluted and concentrated sea water, magnesium chloride, atropin, sodium hydrate and ammonia. He believed that depressants merely hasten the onset of that distinct physiological state, usually accompanied by a negative reaction to light, which larvae normally attain as they grow older. Esterly (1919) found surface specimens of *Acartia* to be negatively geotropic in diffuse light but positive in darkness at the same temperature, although deep sea specimens (at room temperature) showed no change in geotaxis. Marcus (1926a) found that the larvae of *Plumatella fungosa*

are negatively geotactic in nature, both in darkness and in light, throughout the entire natatory period.

In a series of experiments, larvae of *B. neritina* were placed in stender dishes at room temperature and removed to a darkroom for a period of half an hour. When the containers were again returned to diffuse daylight, the larvae showed no change in their reaction to light. Neither was there any change in geotaxis. Similarly, larvae kept at reduced temperatures showed no change of tropisms, when they were returned to diffuse daylight after a 30-minute period in the darkroom.

On the other hand, there is some evidence that darkness reduces the activity of the larvae and decreases the length of the free-swimming period. Three experiments were performed by subjecting larvae to conditions that, in diffuse daylight, ordinarily prolong the natatory period well beyond 2 hours. Two vials, containing sea water diluted to a density of 1.010 grams/cc., were placed in the darkroom. When the first vial was inspected at the end of an hour, all the larvae were found to be active; but at the end of 2 hours only 28 per cent were active, whereas 55.8 per cent were still swimming in the controls. (For the effects of diluted sea water see Table V.) In the second vial none were active at the end of 2.5 hours, although 27.5 per cent of those in the controls were still motile at the end of 4 hours. The third vial contained normal sea water which was kept at a temperature reduced sufficiently to cause prolonged activity in diffuse daylight. None of the larvae in this vial were active at the end of an hour. Although more experiments are needed, the results indicate that darkness probably reduces the length of the natant period in this species.

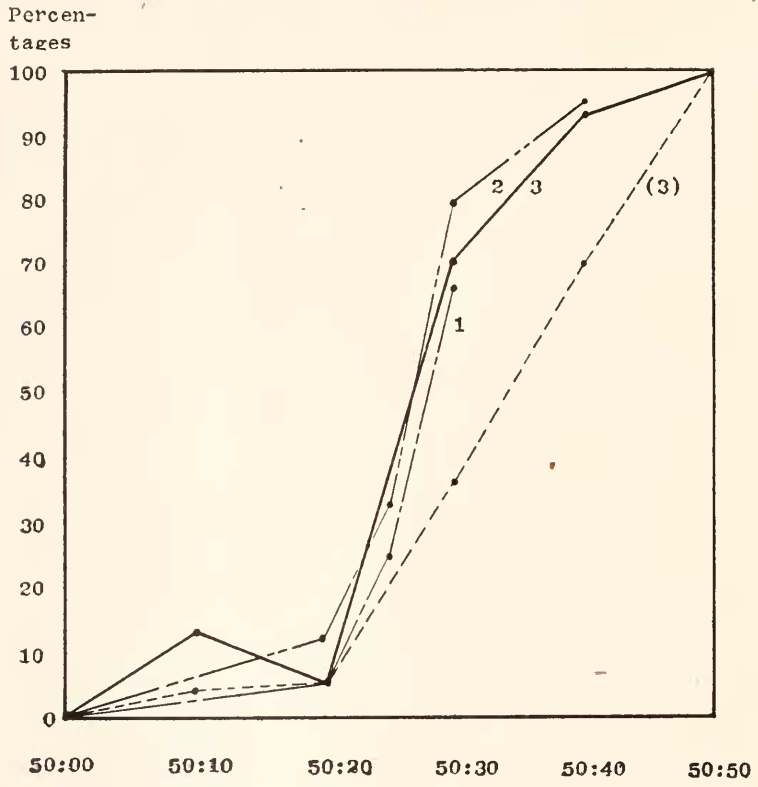
The effects of salinity on the duration of the free-swimming period and the responses of the larvae to light and gravity

Since *Bugula* grew profusely near the laboratory at the mouth of the Newport River, it was surprising that adult colonies could not be detected near the water line on pilings and docks only three-quarters of a mile up-stream. The few that were found were growing near the bottom of objects that could be raised to the surface. A sample of the water in this region, taken about an hour after the tide had turned and sea water had begun running up-stream, was found to have a specific gravity of 0.0116 (28° C.), whereas the lowest of 25 tests made on water near the laboratory gave a reading of 1.0164.[2] It seemed likely that this difference in density, although small, would not represent the maximum, since at low tide the amount of fresh water would undoubtedly be greater and the density would be correspondingly lower.

That the salt content of the water might affect the geotropic responses of the larvae and determine their ultimate place of attachment seemed to be the only explanation for the observed distribution. Furthermore, on several occasions during the summer of 1944 the writer had noticed that larvae seemed to metamorphose in an abnormally short time when concentrated sea water was added to a well-slide. Consequently, a series of experiments were performed by using various concentrations of sea water below and above the normal range. Since 16 out of 25 samples of sea water in the region of the laboratory showed a specific gravity of 1.0200–

[2] In order to avoid unwieldly decimals in Table V, temperature corrections were not made. The maximum deviations due to temperature fluctuations would not exceed 0.00076 gr./cc.

1.0226, this may be considered approximately the normal range during the time the experiments were performed. These samples were taken on different days at both high and low tide during a three-week period. The highest reading was 1.0226 and the lowest was 1.0154; seven readings showed a prevailing density of 1.0164–1.0173 gr./cc. during a period of especially heavy rainfall in the third week of August. Gutsell (1930), however, found a higher average density during the



Graph I. Dilutions of sea water (50 cc.) with distilled water

Lines 1 and 3 (solid) show the percentages of geopositive larvae at the end of 1.5 hrs. Line 2 (solid) shows the number geopositive at 5.5 hrs. Dotted line (3) shows the number active at the end of 1.5 hrs. in vials used for solid line 3.

years his readings were taken (1924–28), even if temperature corrections are made for those listed above; at that time the salinity in this region approximated 30 parts per mille, but occasionally ran as high as 38 or as low as 6 parts per mille (20° C.).

Graph I shows the results of 11 experiments carried out by diluting 50 cc. of sea water with 10 to 50 parts of distilled water. The temperatures were read at the time the densities were taken, and the observations were carried out on the same day between 11 A.M. and 4:30 P.M. (E.S.T.). The following table shows the densities in gr./cc. of the various dilutions used. (The writer found that the

density of a sample of sea water rose approximately 0.00017 gr./cc. for each degree (C.) drop in temperature.) It will be noted that dilutions of 50:10 and 50:20 had but little effect on the number of larvae that became geopositive. The number of geopositive larvae began to increase at dilutions greater than 50:20 and grew progressively larger as the salinity decreased. A decrease in salinity likewise increased the average length of the natatory period. Since the water three-quarters of a mile upstream had a density of 1.0116 gr./cc., a dilution slightly greater than 50:30, its low salt content may explain the location of *Bugula* colonies near the bottom of the stream.

TABLE IV

Dilution	Temperature	Gr./cc.
50:50	29.0	1.0101
50:40	29.5	1.0108
50:30	29.9	1.0119
50:20	30.0	1.0149
50:10	30.0	1.0169
50:00	29.7	1.0228

Since random samples of the laboratory supply of sea water, taken from the mouth of the Newport River, showed fluctuations caused not only by high and low tide but also by the amount of rainfall, more extensive data were obtained by using sea water diluted or concentrated to a definite specific gravity as determined by means of a very sensitive hydrometer. Care was taken to fill the vials to the same level (about 5.5 cm.) and to add approximately the same amount of water, when larvae were pipetted from the finger-bowls. The addition of such small amounts of water to the vials did not seriously increase the densities in the lower ranges, where experimental results were most pronounced. The introduction of non-motile larvae, which cannot respond to treatment, was avoided by first transferring larvae from the finger-bowls to a stender dish of fresh sea water before introducing them into the vials. Since the active ones invariably swam to the lighted side of the dish within a minute or two, large numbers could be transferred without difficulty. Because quiescent larvae sometimes become active after a few minutes of observation, counts had to be made 3 or 4 times for each vial. The difficulty of determining the number of larvae that were active at a given time necessitated the use of only small populations for each vial; the ideal number was found to be about 20 to 30. For these experiments 2,358 larvae were counted during a 5-week period. The results are summarized in Table V.

Larvae kept in water having a specific gravity of 1.010 to 1.012 were extremely active for an abnormally long time, definitely photopositive during the time of greatest activity and geonegative during the early stages of the natatory period. The majority, however, descended to the bottom towards the end of the free-swimming period. These larvae were visibly smaller than those that had a shorter period of activity and measured only 0.14 mm. in diameter as contrasted with the average size, 0.20–0.22 × 0.27–0.30 mm. Since this decrease in volume was found to be correlated with a prolongation of the free-swimming period, it seems likely that the activity of the larvae resulted in a conversion of stored food material into

waste products of metabolism that passed out into the surrounding medium. It is not improbable that the lipoid granules observed by Barrois (1877) and others constitute a reserve food supply for those forms of bryozoan larvae without an alimentary tract. Furthermore, these larvae had extruded an orange colored pigment that gave a characteristic color to the water in the bottom of the vials.

TABLE V

Specific gravity	Number of larvae	Percentage geonegative in hours				Percentage intermediate				Percentage geopositive				Percentage active					
		½	1	2	4	½	1	2	4	½	1	2	4	½	1	2	4	Number larvae	6
1.010	203	44	30	8	6	18	28	38	6	38	42	54	88	100	87	67	39	44	16
1.011	126	72	42	34	22	8	32	36	10	20	26	30	68	97	88	52	38	26	8
1.012	135	85	76	77	65	5	6	6	5	10	18	17	30	87	55	38	27	39	10
1.013	207	98	92	83	58	0	0	0	7	2	8	17	35	59	55	30	9	54	5
1.014	85	96	94	93	86	3	2	2	1	1	4	5	13	66	26	10	7	43	9
1.015	137	88	89	90	93	11	4	4	0	1	7	6	7	65	31	4	1	44	0
1.016	168	84	87	90	90	7	4	1	1	9	9	9	9	28	10	3	0	96	0
1.017	143	88	89	89	90	2	2	2	1	10	9	9	9	26	9	4	2	187	3
1.018	112	92	92	93	93	1	1	0	1	7	7	7	6	32	7	2	1	32	0
1.019	148	98	97	97	97	0	0	0	0	2	3	3	3	12	2	0	0	85	0
1.020	159	95	98	96	96	2	1	1	0	3	1	3	4	21	6	1	1	26	0
1.021	171	97	90	94	93	1	3	3	2	2	7	3	5	19	5	3	1	95	2
1.022	97	95	—	—	94	0	—	—	0	5	—	—	6	10	5	0	0	63	0
1.023	104	83	85	86	88	10	5	5	3	7	10	9	9	25	5	2	0	62	0
1.024	115	69	66	65	65	11	11	12	13	20	23	23	22	30	9	1	0	43	0
1.025	214	15	16	16	16	12	10	11	11	73	74	73	73	8	3	1	0	57	0
1.026	134	7	5	6	6	6	10	7	4	87	85	87	90	13	9	3	2	39	0
Total	2358																		

Temperature of sea water for above—25–29°C.
Temperature at which hydrometer readings were taken—27.5–29°C.

The larvae themselves were orange-brown, whereas those kept in water of higher specific gravities always appeared black. Lillie (1909) found a similar pigment in the larvae of *Arenicola;* this, he said, is derived from the egg cell and normally disappears during later larval stages. Prolonged muscular activity caused this pigment to leave the cells and color the surrounding medium with a faint yellow tinge.

Larvae placed in sea water at densities between 1.016 and 1.022 gr./cc. (the normal range) behaved quite differently from those in the lower densities. An average of 92.1 per cent remained geonegative at the end of 4 hours, and 90 per cent at the time of fixation; in vials containing sea water at a density of 1.010 gr./cc., however, only 6 per cent were geonegative at the end of 4 hours and at the end of the free-swimming period. Furthermore, those kept in sea water at densities between 1.016 and 1.022 gr./cc. had considerably abbreviated natatory periods when contrasted with larvae in water at lower densities. At the end of 4 hours the average percentage of active larvae in 44 vials containing water at the higher densities (1.016–1.022) was 0.71 per cent whereas 34 per cent were active at densities from 1.010 to 1.012 gr./cc. inclusive.

In sea water more concentrated than 1.022 gr./cc. the percentage of larvae that descended to the bottom increased with the density. In these cases, however, a geopositive reaction was not preceded by a prolonged period of activity. At densities of 1.025 and 1.026 gr./cc. only 10.5 per cent were active at the end of half an hour, whereas 22.5 per cent were active in concentrations between 1.016 and 1.024 gr./cc. and 100 per cent were active at a density of 1.010 gr./cc. At a density of 1.026 gr./cc. only a few larvae were active after 10 minutes; they sank passively to the bottom almost as soon as they were introduced into the vials, and generally only feeble movements could be detected afterwards. Evidently the factors that caused these larvae to descend to the bottom were quite different from those that brought about a similar positive geotaxis in sea water of low salt concentration. In the latter, the larvae swam slowly to the bottom after they had been active a very long time. When the effects of concentrated sea water were observed under the microscope, it was found that larvae metamorphosed in a remarkably short time in almost epidemic proportions. It seems unlikely that the organisms were effected by any change in the pH produced by diluting or concentrating the medium, for subsequent tests showed that a dilution to 1.010 gr./cc. caused the pH to drop only 0.08 and a concentration to 1.026 increased the pH only 0.52 ± 0.1.

The development of larvae at various salinities

Vials containing larvae that had already formed zoöids were inspected at the end of 24 hours. Those in more concentrated sea water were slightly larger than normal. All of them had their tentacles everted and snapping actively at this time. These zoöids were attached securely to the substrate.

Those in vials containing sea water at specific gravities of 1.016 and 1.017 were normal in every respect at 29 hours, except that they did not withdraw their tentacles as a reaction to mechanical jarring, as they normally do in higher salt concentrations. Those that developed at a density of 1.015 gr./cc. had their tentacles everted, but these were protruding at an angle of 90° to the normal position. Apparently these organisms were entirely incapable of withdrawing them, for mechanical jarring had no effect during the 30 minutes they were under observation.

In vials containing sea water at densities below 1.015 gr./cc. development was poor. There was a marked reduction in size, and none had their tentacles everted (29 hours). Nevertheless, even those that metamorphosed in water having a density of 1.010 gr./cc. had normally formed internal organs that moved spasmodically at times, and most of them had a bud for the formation of a second zoöid. Again it seems likely that the poor development of these zoöids can be attributed to an insufficiency of stored food at the time of metamorphosis, caused by their prolonged activity. Apparently some of the larvae in water at a density of 1.010 had failed to metamorphose; and many of them did not adhere to the bottoms of the vials, for they could be easily dislodged by a stream of water.

Salinity vs. osmotic pressure

In order to determine whether the observed effects were due to the salt content or to the osmotic pressure, sea water was diluted to a density of 1.010 gr./cc. and sucrose was added until the specific gravity reached 1.026. Since larvae in sea water at a density of 1.010 gr./cc. remained active for an unusually long time, it was considered logical to conclude that osmotic pressure would not be a factor, if the

larvae showed the same behavior after the pressure had been raised by sucrose. On the other hand, if an abbreviation of the natatory period took place, it could be concluded that this effect was caused by an increase in osmotic pressure.

Since subsequent tests showed that similar sucrose solutions had a freezing point depression equal to that of sea water at a density of 1.014 (see Table VI), vials con-

TABLE VI

Density in gr./cc. of original sea water	Substance added	Final density gr./cc.	Freezing point depression	pH
1.010	NaCl	1.017	1.74	7.6 ±.10
1.010	$CaCl_2$	1.017	1.36±.02	7.07±.07
1.010	KCl	1.017	1.67±.11	7.61±.05
1.010	$MgC._2$	1.017		7.58±.14
1.020	$CaCl_2$	1.026	2.20	7.85
1.010	Sucrose	1.026	1.25±.02	7.2 ±.20
1.010	Sucrose	1.017	—	6.7
1.010	Glucose	1.026	1.41	6.2
Distilled	NaCl	1.026	—	6.3
1.017	None	1.017	1.51±.07	7.67±.02
1.024	None	1.024	2.06±.03	8.25
1.026	None	1.026	2.21±.07	8.25±.05
1.010	None	1.010	.96	7.60

± indicate repeated experiments.

Osmotic pressures of sea water at various concentrations. Temperature = 27.5–29.0° C.

Specific gravity	Freezing point depression	Pressure in atmospheres
1.010	0.96	11.56
1.011	1.09	13.12
1.012	1.11	13.36
1.013	1.17	14.08
1.014	1.26	15.16
1.015	1.36±.03	15.36±.36
1.016	1.46±.07	17.56±.84
1.017	1.51±.07	18.16±.84
1.018	1.62±.01	19.48±.12
1.019	1.62±.01	19.48±.12
1.020	1.67±.05	20.08±.60
1.021	1.87	22.48
1.022	1.87	22.48
1.023	1.95	23.44
1.024	2.06±.03	24.75±.35
1.025	2.10±.02	25.23±.25
1.026	2.21±.07	26.55±.96

± indicate repeated experiments.

taining water at this density may be considered controls. In the sucrose solutions 45.3 per cent of the larvae were active at the end of 4 hours and 94 per cent had become geopositive; in the controls only 6.9 per cent were active and 13 per cent had descended to the bottom. It seems apparent that an increase in osmotic pressure by a non-electrolyte does not have the same effect as the addition of ions normally pres-

ent in sea water. These results are in agreement with the observations of Loeb (1900), for he found that marine organisms are easily affected by a disturbance in ionic balance, but are practically independent of osmotic pressure.

Larvae placed in a similar solution of glucose and sea water behaved quite differently, for they immediately sank to the bottom and remained immobile. When the pH values were obtained for similar solutions made with the same glucose, it was found that they were somewhat acid when freshly made (5.8–6.2); by the end of 24 hours the pH had fallen to 4.5, and by 48 hours had reached 3.4. Since the solutions used at Beaufort were made the day before the experiments were performed, presumably the anomalous behavior of the larvae can be attributed to an originally high degree of acidity, which was further enhanced by bacterial action. The sucrose solutions, however, showed no great acidity, for they dropped to only 6.5 at the end of 24 hours. The effect of pH on the behavior of other species of larvae investigated recently at Woods Hole will be described in another paper.

The effects of various ions on the length of the free-swimming period and the metamorphosis of the larvae

Since the experiments just described gave presumptive evidence that variations in salt content played a far more important role than an accompanying increase or decrease in osmotic pressure, the following observations were made to determine the specific effects of the four ions most abundant in sea water by using the chlorides of the metals. For comparing the effects of normal sea water with sea water having a high concentration of only one of the ions, a "fundamental solution" was made by diluting sea water to a specific gravity of 1.010. Since this concentration contains ions in the same proportion as normal sea water and is capable of supporting prolonged larval life, it may be presumed that any modification of the behavior of larvae can be attributed to the ions that were added. The osmotic pressures and pH values of similar solutions are given in Table VI.[3]

Effects of sodium chloride. Larvae placed in pure solutions, made by adding sodium chloride to distilled water until a specific gravity of 1.026 was obtained, sank to the bottom immediately, became immobile and turned completely white within three minutes, so that they could be seen easily only against a black background. When viewed under the microscope, the larvae were observed to undergo metamorphosis very rapidly. Their white appearance was due largely to the enormous amount of milky white holdfast material ejected and to an inward migration of pigment granules from the surface of the larvae. After the initial stage of metamorphosis had begun, the succeeding stages occurred somewhat more slowly than in normal sea water. Most of the larvae were shrunken and appeared to be about two-thirds normal volume. At the end of 3 hours they looked like irregular pieces of white jelly; their edges were ragged and protoplasmic threads protruded from their surfaces. Those on the bottom of the vials were not anchored securely and could be easily disloged by directing water from a pipette against them; and, after being dislodged, they floated to the top. Evidently metamorphosis had decreased their density. Similar results were obtained with solutions made by adding enough sodium chloride to distilled water to raise the specific gravity to only 1.010, but in this case

[3] Densities were measured in connection with ion effects because equipment was easily available for such measurements and these effects were to be compared with other results in which density measurements had been used.

the larvae did not take on a characteristic white appearance until 30 minutes had elapsed. Metamorphosis, therefore, did not occur so rapidly.

Finally a "fundamental solution" was made and raised to a specific gravity of 1.017 by sodium chloride. When first admitted to the vials, the larvae sank to the bottom immediately; but after a few minutes almost all had ascended to the top and were swimming actively. At the end of an hour 16 per cent were still active and 72 per cent had descended to the bottom and remained there, whereas in normal sea water of the same osmotic pressure only 5 per cent were active and 3 per cent were geopositive. The first reaction of larvae placed in a new environment seems to be what Marcus (1926a) called a "schreckreaktion"; they sink down passively and remain motionless for a few minutes. This reaction, when observed under the microscope, was found to be preceded by a violent constriction of the organisms in the region of the equator. Similar behavior was reported for the nauplii of *Balanus amphitrite* by Edmondson and Ingram (1939).

Effects of potassium chloride. The first solution was made hypertonic to the medium from which the larvae were taken by adding potassium chloride to normal sea water (density, 1.020 gr./cc.) until a specific gravity of 1.026 was obtained. The second was made isosmotic to sea water having a density of 1.020 by adding the chloride to a "fundamental solution." In both solutions the larvae sank immediately to the bottom and became milky white as they left a trail of black pigment granules behind them. After reaching the bottom, a few remained slightly active for about 10 minutes. They were somewhat more active in the second solution of lesser density, moving slowly and aimlessly on the bottom, although the cilia were beating in an almost normal manner. Perhaps the effective stroke was reduced enough to prevent active movement. After an hour their appearance was similar to those in the pure solutions of sodium chloride, except that metamorphosis did not occur. Potassium chloride had a similar effect in causing the loss of pigment from already metamorphosed larvae that were floating on the surface of finger-bowls, but in this case the pigment streamed out below the larvae and sank to the bottom; the larvae themselves remained on the surface. Lillie (1909) observed a similar loss of pigment in *Arenicola* larvae placed in potassium solutions (cf., also, Chambers and Reznikoff, 1926).

Effects of calcium. Larvae placed in distilled water raised to a specific gravity of 1.010 by calcium chloride went to the bottom and ceased movement abruptly. At the end of an hour, they looked orange-brown and flattened like oblate spheroids, caused by a shortening of the oral-apical axis; they closely resembled larvae that had a prolonged natatory period in sea water reduced to a density of 1.010 gr./cc., except that those in the calcium chloride solution were larger than normal, measuring 0.29–0.30 × 0.30–0.34 mm. When other larvae were placed in a solution made hypertonic to the medium from which they were taken (sea water at a specific gravity of 1.020) by the addition of calcium chloride until a density of 1.026 gr./cc. was reached, they likewise sank to the bottom but remained slightly active for 2 hours. The cilia and vibratile flagella continued to beat rapidly, even though the larvae themselves did not move. Although the holdfasts had been ejected in many of them, normal attachment did not take place and the larvae continued to move aimlessly around with their holdfasts trailing behind them. They were badly fragmented and considerably reduced in size, measuring only 0.18–0.19 mm. along their

longer diameter. Finally, calcium was added to the "fundamental solution" to bring the specific gravity up to 1.017. Larvae in this solution showed a marked prolongation of the free-swimming period. In one vial 20 per cent were still active after 8 hours, In another, 2 larvae were still active after 12 hours, a strikingly long time in comparison with the normal period of 30–60 minutes. These larvae, in contrast to those in isosmotic sea water (specific gravity, 1.015), behaved quite differently from the latter. Not only did they have a decidedly longer natatory stage, but all, without exception, became geopositive at the end of the free-swimming period; only 8 per cent of those in isosmotic sea water became similarly geopositive. These larvae in the calcium chloride solutions behaved like those in normal sea water diluted to a density of 1.010 gr./cc. Like the latter, they remained geonegative, swimming just under the surface, during the greater part of their larval period, before they descended to the bottom. There was one significant difference, however, for they remained active much longer than those in diluted sea water. Furthermore, they did not attach rigidly like the latter.

Magnesium chloride. Sea water diluted to a density of 1.010 gr./cc. was raised to a specific gravity of 1.017 by magnesium chloride, so as to approximate the osmotic pressure of normal sea water. Larvae in this solution showed an initial shock reaction and sank to the bottom. They recovered rapidly, however, swam to the top and continued to swim feebly at the surface for half an hour. They showed a pronounced tendency to coalesce in groups. Lillie (1909), who observed a similar coalescence of the larvae of *Arenicola* in high concentrations of magnesium chloride, attributed this behavior to a loss of muscular contractility without an accompanying decrease in ciliary movement. By half an hour, active swimming movements had ceased. Whenever larvae at the surface became quiescent, jarring the vials caused them to become active again. Each time this was repeated, several larvae swam downward, after a brief period of swimming at the top, and remained on the bottom. By the end of 40 minutes, all the larvae had descended to the bottoms of the vials. Although they were not swimming, these larvae lying inert on the bottom had failed to attach and metamorphose. Five hours and 45 minutes after they were placed in these solutions, pipettes-full of larvae were removed and added to normal sea water; the majority began to swim actively again.

Mechanical agitation

Although the vertical distribution observed by McDougall (1943) could not be attributed to modifications of any of the factors thus far investigated, since they had to be more extreme than any prevailing in nature, nevertheless one correlation became increasingly evident. Prolonged activity of the larvae was always associated with a positive geotaxis. This was true, whether induced by a reduction of temperature or salinity or by an excess of calcium over the other ions present in sea water. Furthermore, mechanical jarring, by activating the larvae in solutions of magnesium chloride, had caused them to descend to the bottom. Agitation by waves and wind seemed to be the stimulus provided by nature for bringing about a positive geotaxis. Moreover, experiments on other animals have shown a correlation between mechanical agitation and a change in physiological state. Rogick (1939), for instance, found that excessive handling of the larvae of fresh-water bryozoans abnormally prolonged and interfered with metamorphosis. Similarly, contact

stimuli frequently change the phototropism of organisms, as evidenced by the experiments of Holmes (1905) on *Ranatra*, Parker (1902) on *Labidocera* and Towle (1920) on *Cypridopsis*. The writer likewise observed that the larvae of *B. neritina* became negatively phototropic for about a minute after they were ejected from a pipette.

To determine the effect of mechanical agitation on larval behavior, an air jet from a pipette was directed against the surface of the water whenever larvae showed a tendency to become quiescent. This caused them to swim actively again. In brief, 19 per cent were active and 62.5 per cent were geopositive in the experimental vial at the end of 4 hours, whereas only 11 per cent were active and 11 per cent were geopositive in the control. In view of the correlation that exists between mechanical agitation and swimming movements on the one hand and between prolonged activity and a positive geotaxis on the other, it seems likely that further experiments would yield similar or even better results. Since the writer never observed that larvae were liberated in darkness under laboratory conditions, it is difficult to explain the observation that in nature they attach as readily at night as in the daytime (Edmondson and Ingram, 1939; Edmondson, 1944), unless it is assumed that these organisms, liberated by light, remain active through mechanical agitation during the day and part of the night. Certain experiments performed by the writer indicate that light, as well as mechanical agitation, may serve as a stimulus to movement.

Discussion

The nature of geotaxis in B. neritina

Mechanical agitation is one of the factors existing in nature that is not generally duplicated under laboratory conditions, even though all the other factors belonging to the habitat of an organism seem to be present. The abnormally tranquil surfaces prevailing in the laboratory are not at all comparable to those encountered by larvae when they reach the surface of the ocean and are constantly buffeted about by waves and wind. Presumably larvae in their natural surroundings have a somewhat longer free-swimming period than those under laboratory conditions. The action of waves and wind prevents them from attaining that degree of quiescence which is a natural prerequisite for metamorphosis. Thus they remain active; and, as a result of prolonged activity, they descend to regions where a greater degree of calmness prevails. Crevices in rocks and pilings undoubtedly afford a certain amount of protection against the buffeting action of waves; hence a natural explanation may be given for the preponderance of young zoöids that can be observed in grooves and in holes bored into test panels, after they have been submerged for a few days. At the present time there cannot be given a completely empirical explanation that will clearly demonstrate the *mŏdus operandi* of the various factors that bring about a positive geotaxis under natural conditions.

Geotaxis in marine larvae cannot be assigned to the same category of responses as the other tropisms. Phototropism, for instance, whether it be positive or negative, is an active kind of response. On the other hand, what we call a positive or negative geotaxis can be resolved into other mechanisms. A negative geotaxis can be attributed to ciliary action, to buoyancy or to a more abundant supply of oxygen near the surface. It has sometimes been identified with a positive phototropism. Many aquatic larvae, such as those of *Arenicola* (Lillie, 1901) or of the phylacto-

laematous Bryozoa (Marcus, 1926c), are specifically denser than the surrounding medium. They can keep afloat only by ciliary movement, and when the cilia are injured they sink to the bottom. Others, apparently, are lighter than the surrounding medium and are buoyed up passively by Archimedes' Principle. Hora (1930) stated that some organisms can decrease their specific gravity by a reduction of the abdominal cavity; others, such as the larvae of *Megalophrys,* have hydrostatic organs. Thus their rising or sinking depends upon their density. Similarly, Visscher (1928) observed that the cyprid larvae of *Balanus eburneus* have a fatty substance in the anterior of their bodies that acts like a buoy in holding them near the surface; this lipoid material gradually disappears towards the end of the pelagic period. McDougall (1943) found that the setting of these larvae occurred in greatest abundance at a point some 5 or 6 feet below the surface. In another species, *Chthamalus fragilis,* however, he found that the lipoid droplets do not disappear towards the end of the free-swimming period; the highest incidence of settings of this species was very near the surface, and no attached larvae were found more than 6 inches below mean low water. Evidently buoyancy, or the lack of it, can determine the vertical distribution of certain organisms. In the cyprid larvae just described phototropism is not a factor in determining the ultimate place of fixation, for Visscher (1928) found that both species are photonegative just before attachment. A positive geotaxis should not be confused with a negative phototropism, as has been done sometimes in the case of bryozoan larvae. Sometimes, at least, the two tropisms are dependent upon entirely different mechanisms.

There are several possible explanations for differences in the behavior of the larvae of *B. neritina* in warm and cold water. It might be assumed that, since oxygen would be more abundant near the surface, the larvae would collect there when the temperature is high, because they are unable to obtain sufficient oxygen to support their increased metabolic activity, if they venture far from the surface film. The possibility that a negative geotaxis may be simply a positive response to oxygen in the case of free-swimming larvae has been suggested by Marcus (1926a) as a plausible explanation for the pelagic habits of certain marine Bryozoa. In his experiments on fresh-water Bryozoa (1926c), however, he could not obtain conclusive evidence that the larvae move in the direction of an increasing oxygen gradient. The writer, also, found no confirmation of such a theory. When a vial of sea water was inverted over a petri dish with the bottom supported on two slides so that oxygen could enter only from below, the larvae showed not the slightest tendency to move downward. Neither did they surround an air bubble that was on the side of the vial opposite the source of illumination. Furthermore, it would be difficult to explain a positive response to gravity, when the temperature is lowered, on the assumption that the negative geotaxis exhibited in warm water is merely a positive response to oxygen. Even though the oxygen content of water at reduced temperatures might be sufficient to maintain the lowered metabolic requirements of larvae at various depths beneath the surface, their movement in a direction of a slightly decreasing oxygen gradient would require explanation.

At first sight, it would seem logical to attribute the rising or sinking of the larvae to ciliary action alone, if it is assumed that these organisms are denser than the surrounding medium. Their descent in hypotonic solutions only after prolonged swimming might be attributable to ciliary fatigue. Since a reduction in

temperature decreases ciliary movement in the gills of *Mytilus* (Gray, 1923) and since hypertonic solutions (Gray, 1922), sea water having a pH below 5.5–6.0 (Gray, 1920) and solutions of sodium and potassium (Gray, 1920) have an even more deleterious effect, the sinking of the larvae of *B. neritina* under almost identical conditions might seem to be due solely to attenuation of ciliary action. The prolonged negative geotaxis of these organisms in solutions containing calcium concentrated to a certain optimum would seem to be correlated with the favorable effects of this ion on ciliary action, as described by Lillie (1901) for *Arenicola*. On the other hand, it might be expected that ciliary action would be maintained better in solutions containing an excess of potassium ions than in a similar sodium solution of approximately the same pH and osmotic pressure, for the latter has a more pronounced inhibitory effect on the cilia of *Mytilus* (Gray, 1920) or of veliger (Mayer, 1911) or *Arenicola* (Lillie, 1901) larvae.

The larvae of *B. neritina*, although they swim sluggishly in a horizontal plane just beneath the surface film in warm water, give no indication that they remain there only by active swimming. In fact, both dead and metamorphosed larvae were always found floating at the surface, and obviously ciliary action cannot account for this. They seem rather to be buoyed up by the medium. If this is true, they would differ from the phylactolaematous Bryozoa, which maintain themselves at the surface only by active swimming and sink downwards when injured by heat or by other unfavorable conditions (Marcus, 1926c). Again, larvae swam without exception towards the side of a vessel nearer the source of diffuse daylight, whereas only 42.2 per cent swam downward when they were illuminated only from below. This difference in behavior would seem to indicate that ciliary action is much more effective in moving them along a horizontal plane than vertically downwards against what appears to be a buoyancy gradient. Presumably the orientation of their long axes determines their direction of motion. It cannot be assumed, however, that larvae at room temperature do not swim beneath the surface because their apical ends are directed upwards in orientation to light entering obliquely from above, for they remain on the surface in darkness. If the larvae are heavier than the medium, it should be easier for them to swim downward, for the pull of gravity would be added to their ciliary action; consequently, it would be logical to expect that large numbers of larvae kept in darkness and unoriented to light would swim downward and that this response would be universal in the case of larvae illuminated only from below.

A reduction of ciliary action alone does not seem to be an adequate explanation for a positive geotaxis in these larvae; it seems necessary to posit a simultaneous increase in density. Since larvae invariably became geopositive after they had been active for an abnormally long time, it seems probable that their descent to the bottom can be attributed to a hydrostatic principle. The vitelline mass within these organisms may decrease gradually in volume as they grow older and swim longer; and since it is composed largely of fatty globules of low specific gravity, depletion of lipoids and shrinkage of the larvae would decrease their volume and increase their density, thus causing them to descend at the end of the pelagic stage. This view is confirmed by the observation that larvae smaller than average descended to the bottom, whether this condition was attributable merely to normal variations in size or to a visible reduction in volume resulting from an experimentally induced pro-

longation of activity. After prolonged swimming at the surface, larvae were noticeably smaller when they descended to the bottom in solutions of glucose, calcium and hypotonic sea water. In the latter solution it seems probable that they would have swollen somewhat in the beginning and would, therefore, have displaced a greater volume of the medium. They would thus be buoyed up until their volume decreased again through a conversion of food into waste products of metabolism. Since larvae sank passively in hypertonic sea water, they probably lost fluid to the medium, and their subsequent reduction in volume may have made them denser than the solution. Their rapid descent, however, apparently cannot be explained on the principle of osmosis alone. Probably both injury to the cilia and a violent muscular contraction were involved. An initial shock reaction was actually observed under the microscope in larvae exposed to an excess of certain ions; they underwent a violent constriction in the equatorial region, which may have decreased their volume without altering their weight appreciably.

Since du Bois-Reymond (1914) has shown that the same temperature increment will cause a piece of tissue to expand three times as much as a similar volume of water, it seems likely that larvae may sink to the bottom in cold water in a comparatively short time (15–20 min.) because of an increase in density brought about by shrinkage. Similarly the failure of larvae to descend in warm water might be attributed to a swelling of the vitelline mass, which would cause them to displace a greater volume of water; buoyancy would thus hold them just beneath the surface film. Their reaction recalls the behavior of orthotoluidine drops that float on the surface of warm water, but sink to the bottom when the temperature is reduced; like the larvae, these drops, having approximately the same density as water, will rise again when the medium is warmed only a few degrees. What seems to be a downward spiralling movement of the larvae may be merely a succession of circular paths at descending horizontal levels.

Factors influencing metamorphosis

Only conjectures may be offered at this time regarding the effects of various solutions on the onset of metamorphosis. At first, the writer was inclined to assume that sodium itself is a specific agent that brings about metamorphois. The rapid setting of larvae in sodium solutions would suggest this hypothesis. Furthermore, sodium would presumably favor the violent muscular contraction necessary for the ejection of the holdfast. The fact that both calcium and magnesium either prevented metamorphosis or delayed it far beyond the normal time of onset might be interpreted on the basis that these ions decreased the permeability of the larval tissues, so that sodium could enter only with difficulty. Concomitance of events, however, suggests but does not prove a causal relationship. In view of the fact that there seem to be many substances capable of inducing metamorphosis in ascidians (Berrill, 1930; Zinkin, 1938), it would not be illogical to predict that the same may be true of bryozoan larvae. If metamorphosis is essentially a process of dedifferentiation, as Huxley (1922) has suggested, it is quite possible that any inhibiting agent can bring it about. Copper is capable of inducing metamorphosis in the oyster (Prytherch, 1934) and has a similar role in ascidian larvae (Grave, 1941; Berthold and Mast, 1944). Miller (1946) reported that copper has some effect in hastening the onset of metamorphosis in bryozoan larvae, but he did not discuss the

experiments that led him to this conclusion. Consequently, it may be that sodium has no specific effect on the larvae of *B. neritina;* it may have either a direct effect by inhibition, and in this respect it would not be unique, or an indirect action by increasing the permeability of larval tissues to ions, such as copper, that have an oligodynamic action. (The effects of ions, especially sodium and copper, on two species from Woods Hole will be discussed more fully in a future paper.)

Caswell Grave (1936) postulated the existence of a by-product of metabolism in ascidian larvae, which is produced in greater quantity by energetic swimming movements. This later product, he says, unites with a "susceptibility factor" formed by the secretion of some larval tissue or organ in the production of a third substance, which apparently acts directly on the larval nerve centers to cause the progressive series of responses that constitute the process of metamorphosis. In *B. neritina*, however, it seems unlikely that the accumulation of metabolic waste products has any influence in hastening metamorphosis. *A posteriori,* it would be more logical to attribute the prolonged activity of larvae under certain conditions to factors that delay the accumulation or concentration of some substance that effects the onset of metamorphosis.

The writer wishes to express his gratitude to Prof. H. W. Stunkard, New York University, for the criticism, encouragement and inspiration he has given; to Prof. A. S. Pearse, Duke University, who kindly identified the species described in this paper. Dr. H. F. Prytherch, Director of the U. S. Bureau of Fisheries, Beaufort, North Carolina, enabled the writer to use the laboratories during the summers of 1944 and 1945 and offered many interesting observations on fouling. The writer is also indebted to Dr. Frederick McCutcheon, North Carolina State College, for many valuable suggestions regarding experimental technique; to Dr. Daniel Ludwig, New York University, for helpful criticism in preparing the manuscript; to Dr. M. D. Rogick, New Rochelle College, for literature on the Bryozoa and for reading the manuscript; and to Miss Marie Bentivoglio for lending her talent to the execution of some of the figures and tables that appear in this paper.

Summary

1. The distribution, external morphology, swimming movements and metamorphosis of the larvae of *B. neritina* are discussed. During metamorphosis the eye-spots of the larva function in orientation to light.

2. At room temperature (26–30° C.) the larvae under laboratory conditions are geonegative and photopositive during the larval period. Just before metamorphosis they lose their intense positive reaction to light and become more or less indifferent.

3. A reduction of temperature causes larvae to become geopositive and simultaneously prolongs their free-swimming period. When either ascending or descending gradients of temperature are employed, the greater number of larvae change their responses to gravity between 20 and 23.5° C.

4. In cold water (7 to 10° C.) larvae lose their intense positive response to light and become more or less indifferent. Their behavior at reduced temperatures shows that they may become geopositive without exhibiting a simultaneous negative reaction to light. Their descent to the bottom of a vial is not caused by a negative phototropism. Either of the two tropisms may be independent of the other.

5. Larvae made geopositive by a reduction in temperature do not swim upwards towards a source of illumination, when the rays can enter a vial only from above. Neither do they react negatively to light, when the rays enter only from below. Their positive geotaxis cannot be modified by light. At room temperature, however, approximately half the larvae, originally geonegative, swim downwards towards a source of light placed beneath a vial.

6. At 28–30° C. the geotaxis of the larvae is not affected by rapid changes in light intensity.

7. At room temperature, intense light (sunlight and artificial illumination) has some effect in driving larvae to the bottom of a vial.

8. When larvae are returned to diffuse daylight after a 30-minute period in darkness, they do not change either their phototropism or their geotaxis, when they are maintained at low (7–8° C.) or high (26–30° C.) temperatures. Darkness, however, probably reduces their activity and shortens the larval period.

9. A reduction (40–50 per cent) of the salt content of sea water greatly prolongs the natatory period and causes larvae to become geonegative after a long period of swimming at the surface. Hypertonic sea water, however, greatly reduces the free-swimming period, induces a more rapid onset of metamorphosis and causes the larvae to become geopositive. The development of larvae that metamorphosed in sea water of various salinities is discussed.

10. A slight increase in the osmotic pressure of diluted (50 per cent) sea water by the addition of a non-electrolyte does not cause the same response as a similar increase in salt content.

11. An excess of either sodium or potassium causes a rapid loss of pigment. Potassium has the more pronounced effect. Sodium induces a rapid onset of metamorphosis, but potassium does not. Both sodium and potassium cause rapid sedimentation of larvae.

12. A similar excess of calcium greatly prolongs swimming movements and inhibits metamorphosis. An excess of magnesium inhibits metamorphosis, but does not cause prolonged swimming.

13. Mechanical agitation, hypotonic sea water and calcium apparently bring about a positive geotaxis by abnormally prolonging the free-swimming period of the larvae.

14. A tentative explanation of geotaxis in these organisms and a discussion of the effects of various factors on their metamorphosis are presented.

LITERATURE CITED

397

Allmann, G. J., 1872. On the structure of cyphonautes. *Quart. Jour. Micr. Sc.*, **12**: 395–417.

Barrois, J., 1877. Recherches sur l'Embryologie des Bryozoaires. 4to. Lille.

Barrois, J., 1879. Mémoire sur la métamorphose des Bryozoaires. *Ann. des Sci. Nat.* (*Zool.*), Ser. 6, **9**: 1–67.

Barrois, J., 1882. Embryogénie des Bryozoaires. *Jour. Anat. et Physiol.*, **18**: 124–157.

Barrois, J., 1886. Mémoire sur la métamorphose de quelques Bryozoaires. *Ann. des Sci. Nat.* (*Zool.*), Ser. 7, **1**: 1–67.

Berrill, N. J., 1930. II. Studies on tunicate development. Part I. General physiology of development of simple ascidians. *Phil. Transact. Roy. Soc. London,* Ser. B, **218**: 37–79.

Bertholf, L. M., and S. O. Mast, 1944. Metamorphosis in the larva of the tunicate, Styela partita. *Biol. Bull.*, **87**: 166.

Calvét, L., 1900. Contribution à l'histoire naturelle des Bryozoaires ectoproctes marins. Thesis pres. à la Faculté des Sciences de Paris. *Trav. Inst. de Zool.,* N.S. **8**: 22.

Canu, F., and R. F. Bassler, 1929. Bryozoa of the Philippine region. *Smithsonian Inst. U. S. Nation. Mus. Bull. 100,* **9**: 1–685.

Chambers, R., and P. Reznikoff, 1925. Micrurgical studies in cell physiology I. *Jour. Gen. Physiol.,* **8**: 369–402.

Child, C. M., 1925. The axial gradients in Hydrozoa VI. *Biol. Bull.,* **48**: 19–37.

Claparède, E., 1870. Beiträge zur Anatomie und Entwicklungsgeschichte der Seebryozoen *Zeitschr. wiss. Zool.,* **21**: 137–175.

duBois-Reymond, R., 1914. Volumänderungen organischer Gewebe mit Berücksichtigung der "Schwebefauna." *SB Ges. Freunde.,* Jg. **8**: 373–378.

Edmondson, C. H., 1944. Incidence of fouling in Pearl Harbor. *Occas. Papers Bishop Mus.,* **18**: 1–34.

Edmondson, C. H., and W. M. Ingram, 1939. Fouling organisms in Hawaii. *Occas. Papers Bishop Mus.,* **14**: 251–300.

Esterly, C. O., 1919. Reactions of various plankton animals with reference to their diurnal migrations. *Univ. Cal. Publ. Zool.,* **19**: 1–83.

Grave, B. H., 1930. The natural history of Bugula flabellata at Woods Hole, Massachusetts, including the behavior and attachment of the larva. *Jour. Morph.,* **49**: 355–383.

Grave, Caswell, 1936. Metamorphosis of ascidian larvae. *Papers from Tortugas Lab.,* **29**: 211–291.

Grave, Caswell, 1941. Further studies of metamorphosis of ascidian larvae. *Biol. Bull.,* **81**: 286–287.

Gray, J., 1920. The effects of ions on ciliary movement. *Quart. Jour. Micr. Sci.,* **64**: 345–372.

Gray, J., 1922. The mechanism of ciliary movement I and II. *Proc. Roy. Soc. London,* Ser. B, **93**: 104–132.

Gray, J., 1923. The mechanism of ciliary movement III. *Proc. Roy. Soc. London,* Ser. B, **95**: 6–15.

Gutsell, J. S., 1930. Natural history of the bay scallop. *Bull. U. S. Bureau of Fisheries,* **46**: 562–632.

Harmer, S. F., 1885. The structure and development of Loxosoma. *Quart. Jour. Micr. Sci.,* **25**: 261–296.

Harmer, S. F., 1887. Sur embryogénie des Bryozoaires. *Archiv. de Zool. Exp.,* Ser. 2, **5**: 443–557.

Harmer, S. F., 1903. On the morphology of the Cheilostomata. *Quart. Jour. Micr. Sci.,* **46**: 263–351.

Harmer, S. F., 1931. Recent work on the Polyzoa. *Proc. Linn. Soc. London,* **143**: 113–168.

Hatschek, R., 1877. Embryonalentwicklung und Knospung der Pedicellina echinata. *Zeitschr. wiss. Zool.,* **29**: 502–550.

Hillen, E. J., 1923. Report to the bureau of construction and repair. *U. S. Navy Dept.* Nov., 1923.

Hinks, T., 1880. A history of the British marine Polyzoa. Vol. I. CXLI. 601 p. London.

Holmes, S. J., 1905. The reactions of Ranatra to light. *Jour. Comp. Neur. Psychol.,* **15**: 305–349.

Hora, S. L., 1930. Ecology, bionomics and evolution of the torrential fauna, with special reference to the organs of attachment. *Phil. Trans. Roy. Soc.,* Ser. B, **218**: 171–283.

Huxley, J. S., 1922. Dedifferentiation in Echinus larvae, and its relation to metamorphosis. *Biol. Bull.,* **43**: 210–238.

Joliet, L. M., 1877. L'histoire naturelle des Bryozoaires des côtes de France. *Archiv. de Zool. Exp.,* Ser. 1, **6**: 193–296.

Kupelwieser, L. M., 1905. Untersuchungen über den feineren Bau und die Metamorphose des Cyphonautes. *Zoologica,* **47**: 1–50.

Lillie, R. S., 1901. On differences in the effects of various salt solutions on ciliary and on muscular movements in Arenicola larvae I. *Amer. Jour. Physiol.,* **5**: 56–85.

Lillie, R. S., 1909. On the connection between changes in permeability and stimulation and on the significance of changes in permeability to carbon dioxide. *Amer. Jour. Physiol.,* **24**: 15–45.

Lillie, R. S., 1911. The relation of stimulation and conduction in irritable tissues to changes in the permeability of the living membrane. *Amer. Jour. Physiol.,* **28**: 197–223.

Loeb, J., 1900. On ion-proteid compounds and their role in the mechanics of life phenomena I. The poisonous character of pure NaCl solutions. *Amer. Jour. Physiol.*, **3**: 327–338.

Loeb, J., 1905. Studies in general physiology. *The Decennial Publ. Univ. Chicago,* Ser. 2, (XV) **1**: 265–296.

Marcus, E., 1921. Über die Verbreitung der Meeresbryozoen. *Zool. Anz.*, **53**: 5–35.

Marcus, E., 1926a. Sinnesphysiologie und Nervensystem der Larve von Plumatella fungosa. *Zool. Anz. Suppl.*, **2**: 86–91.

Marcus, E., 1926b. Beobachtungen und Versuche an lebenden Meeresbryozoen. *Zool. Jahrb. Syst.*, **52**: 1–102.

Marcus, E., 1926c. Beobachtungen und Versuche an lebenden Susswasserbryozoen. *Zool. Jahrb. Syst.*, **52**: 279–351.

Massart, J., 1888. Recherches sur les organismes inférieurs, I. La loi de Weber vérifée pour l'heliotropisme du champignon. *Bull. Belgique Acad.*, Ser. 3, **16**: 590–597.

Mast, S. O., 1911. *Light and the behavior of animals.* John Wiley and Sons. New York. 410 p.

Mayer, A. G., 1911. The converse relation between ciliary and neuromuscular movements. *Papers from the Tortugas Lab.*, **3**: 1–25.

McDougall, K. D., 1943. Sessile marine invertebrates at Beaufort, North Carolina. *Ecol. Monog.*, **13**: 321–374.

Metschnikoff, E., 1871. Beiträge zur Entwicklungsgeschichte einiger niederer Thiere. 5 Seebryozoen. *Bull. Acad. Sci. Petersbourg,* **15**: 507.

Miller, M. A., 1946. Toxic effects of copper on attachment and growth of Bugula neritina. *Biol. Bull.*, **90**: 121–141.

Miyazaki, I., 1938. On fouling organisms in the oyster farm. *Japanese Soc. Sci. Fisheries Bull.*, **6**: 223–232.

Nitsche, H., 1870. Beobachtungen über die Entwicklungsgeschichte einiger chilostomen Bryozoen. *Zeitschr. wiss. Zool.*, **20**: 1–37.

O'Donoghue, C. H., 1926. Observations on the early development of Membranipora villosa Hincks. *Contr. Canadian Biol. and Fish.*, **3**: 249–263.

Osburn, R. C., 1914. The Bryozoa of the Tortugas Islands, Florida. *Papers from the Tortugas Lab.*, **5**: 181–222.

Osburn, R. C., 1927. The Bryozoa of Curaçao. *Bijdragen tot de Dierkunde (Amsterdam)*, **25**: 123–132.

Ostroumoff, A., 1885. Note sur la métamorphose du Cyphonautes. *Zool. Anz.*, Jg. **8**: 219.

Ostroumoff, A., 1886. Contribution à l'étude zoologique et morphologique des Bryozoaires du golfe de Sébastopol. *Archiv. Slav. Biol.*, **2**: 22–64.

Parker, G. H., 1902. Reactions of copepods to various stimuli and the bearing of this on daily depth migrations. *Bull. U. S. Fish Comm. for 1901:* 103–123.

Pergens, E., 1889. Untersuchungen an Seebryozoen. *Zool. Anz.*, Jg. 12: 504–511 and 526–533.

Prouho, H., 1890. Recherches sur la larve de Flustrella hispida. *Archiv. de Zool. Exp.*, Ser. 2, **8**: 409–459.

Prouho, H., 1892. L'Histoire des Bryozoaires. *Archiv. de Zool. Exp.*, Ser. 2, **10**: 557–656.

Prytherch, H. F., 1934. The role of copper in the setting, metamorphosis and distribution of the American oyster, Ostrea virginica. *Ecol. Monog.*, **4**: 47–107.

Repiachoff, W., 1877. Über die ersten embryonalen Entwicklungsvorgänge von Tendra zostericola. *Zeitschr. wiss. Zool. Suppl. 1878,* **30**: 25–66.

Robertson, A., 1905. The non-incrusting Chilostomatous Bryozoa of the West Coast of North America. *Univ. Cal. Publ. Zool.*, **2**: 235–332.

Rogick, M. D., 1939. Studies on fresh-water Bryozoa VIII. Larvae of Hyalinella punctata (Handcock, 1850). *Trans. Am. Micr. Soc.*, **58**: 199–209.

Salensky, M., 1874. Untersuchungen an Seebryozoen. *Zeitschr. wiss. Zool.*, **24**: 343–349.

Scheer, B. T., 1945. The development of marine fouling communities. *Biol. Bull.*, **89**: 103–121.

Schmidt, O., 1876. Die Gattung Loxosoma. *Archiv. mikrosk. Anat.*, 12: 1–20.

Schneider, A., 1869. Zur Entwicklungsgeschichte und systematischen Stellung der Bryozoen und Gephyreen. *Arch. mikrosk. Anat.*, **5**: 260–280.

Seeliger, O., 1890. Bemerkungen zur Knospenentwicklung der Bryozoen. *Zeitschr. wiss. Zool.,* **50**: 566–600.

Seeliger, O., 1906. Über die Larven und Verwandschaftsbeziehungen der Polyzoen. *Zeitschr. wiss. Zool.,* **84**: 1–79.

Strasburger, E., 1878. Wirkung des Lichtes und der Wärme auf Schwärmsporen. *Jena Zeitschr.,* N.F., **12**: 551–625.

Towle, E., 1920. A study of the heliotropism of Cypridopsis. *Amer. Jour. Physiol.,* **3**: 345–365.

Verrill, A. E., and S. T. Smith, 1874. Report upon the invertebrate animals of Vineyard Sound and adjacent waters, with an account of the physical features of the region. *Rep. U. S. Comm. Fish and Fisheries (1871–1872)*: 295–544.

Vigelius, W. J., 1886. Zur Ontogenie der marinen Bryozoen. *Mittheil. Zool. Stat. Neapel.,* **6**: 499–541.

Visscher, P. V., 1927. Nature and extent of fouling of ships' bottoms. *Bull. U. S. Bureau Fish.,* **48**: 193–253.

Visscher, P. V., 1928. Reactions of the cyprid larvae of barnacles at the time of attachment. *Biol. Bull.,* **54**: 336–350.

Waters, A. W., 1925. Ancestrulae of Cheilostomatous Bryozoa. *Ann. Mag. Nat. Hist.,* Ser. 9, **15**: 341–352.

Yerkes, R. M., 1900. Reactions of Entomostraca to stimulation by light II. Reactions of Daphnia and Cypris. *Amer. Jour. Physiol.,* **4**: 405–423.

Zinkin, L. N., 1938. Stimulation of metamorphosis in ascidian larvae. *Comptes rendus (Doklady) de l'Acad. des Sci. de l'URSS.,* **18**: 213–216.

ZoBell, C. E., and E. C. Allen, 1935. The significance of marine bacteria in the fouling of submerged surfaces. *Jour. Bact.,* **29**: 239–251.